Generation of Glycolaldehyde from Guinea Pig Airway Epithelial Monolayers Exposed to Nitrogen Dioxide and Its Effect on Sodium Pump Activity.

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Pulmonary injury from nitrogen dioxide (NO_2) may in part be related to the generation of aldehydic compounds, which bind with cellular proteins and subsequently impair or inhibit cell function. We examined the generation of aldehydes from guinea pig tracheobronchial epithelial (GPTE) cell monolayers exposed to NO_2. With the use of dinitrophenylhydrazine (DNP) to derivatize aldehydic compounds, glycolaldehyde, a two carbon α-hydroxyaldehyde, was identified in elevated levels in the basolateral fluid from monolayers exposed to NO_2. DNP-glycolaldehyde levels were 81.2 ± 2.7 and 234.0 ± 42.6 nM in response to a 1-hr exposure to 1 and 5 ppm NO_2, respectively, as compared to an air-control value of 20.3 ± 6.8 nM. Taking into account dilution and reactivity, cellular glycolaldehyde levels could have reached as high as 3 mM for the 60-min exposure period (i.e., 0.05 mM/min). The effects of exogenous glycolaldehyde on GPTE ouabain-sensitive basolateral ^{86}Rb uptake (an index of Na^+,K^+-ATPase activity) were examined and compared with the actions of NO_2 exposure. Bolus addition of glycolaldehyde to the basolateral fluid at concentrations ≥5 mM led to an inhibition of ouabain-sensitive ^{86}Rb uptake, while lower concentrations had no effect. The effects of exogenous glycolaldehyde differ from NO_2 exposure, which led to a sustained elevation of ouabain-sensitive ^{86}Rb uptake with presumed generation of glycolaldehyde at a continuous low level. Glycolaldehyde does not appear to play a significant role in the acute alterations of sodium pump activity, suggesting that the NO_2-induced changes in Na^+,K^+-ATPase activity of GPTE monolayers probably are further mediated by other lipid peroxidation products/oxidation processes yet to be identified. *Key words:* airway epithelium, glycolaldehyde, nitrogen dioxide, ouabain-sensitive ^{86}Rb uptake. *Environ Health Perspect* 104:852–856 (1996)

Nitrogen dioxide (NO_2) is a free radical formed by combustion processes in air. An increased incidence of airway inflammation and reactivity are associated with exposure to elevated indoor levels of this reactive gas (*1–3*). The toxicity of NO_2 has been attributed to the oxidation of cellular constituents and generation of reactive compounds that initiate cellular injury (*4*). Free radical oxidation of cellular constituents such as polyunsaturated fatty acids (PUFA) can lead to the generation of aldehydic compounds. Oxidation of sugars may also be an important source of aldehydic compounds (*5*). These aldehydes may bind with essential cellular proteins to impair or inhibit activity, ultimately leading to adverse effects on cell function and viability. In the present study, we have examined the effects of NO_2, a potent oxidant gas, on generation of aldehydic compounds from airway epithelial monolayers and the actions of a predominant aldehydic product on Na^+,K^+-ATPase (sodium pump), an enzyme that is vital to cellular function and viability.

The predominant reaction of NO_2 may be to react with PUFA by abstraction of an allylic hydrogen (*6,7*). The epithelium lining the lung airways, which possesses a significant content of PUFA (*8*), represents one of the first major targets of NO_2 and receives a large burden of the exposure (*9–11*). The mucin layer overlying airway epithelial cells also possesses a significant content of PUFA, as well as other compounds such as sugars, which may potentially interact with this gas (*12*). Lipid radicals, generated by hydrogen abstraction, in the presence of molecular oxygen initiate free radical chain autoxidation of membrane PUFA (*5–7*). The first product of autoxidation is a lipid hydroperoxide. These hydroperoxides may decompose to form a number of products, including reactive aldehydes (*13*). Aldehydes have longer half lives and possess significant cytotoxicity (*13*).

Cytotoxic effects attributed to aldehydes include depletion of intracellular glutathione; decreased protein thiols; onset of lipid peroxidation; disturbance of calcium homeostasis; inhibition of DNA, RNA, and protein synthesis; and inhibition of cellular respiration and glycolysis (*13–15*). Oxidation products derived from either the mucin layer or the apical airway epithelial cell plasma membrane may bind with cellular proteins and subsequently impair or inhibit cellular function.

In the present study, we have examined the generation of aldehydic compounds from guinea pig tracheobronchial epithelial (GPTE) monolayers exposed to NO_2 levels associated with indoor exposure. GPTE cells were cultured in an air interface to form tight monolayers that actively transport ions and solutes vectorially (*16–19*). These cell monolayers were acutely exposed for 1 hr to NO_2 (1 or 5 ppm) and the release of aldehydes into the basolateral fluid was examined. We have identified glycolaldehyde, a two carbon α-hydroxyaldehyde, released into the extracellular medium from GPTE monolayers exposed to NO_2. We then evaluated the effects of exogenous glycolaldehyde on epithelial Na^+,K^+-ATPase activity through measurements of ouabain-sensitive ^{86}Rb uptake and specific ^3H-ouabain binding and compared its actions with those observed for NO_2 exposure.

Materials and Methods

Materials

^3H-ouabain (specific activity 24 Ci/mmol) and $^{86}RbCl$ (1.54 mCi/mg) were obtained from New England Nuclear (Boston, MA). Glycolaldehyde dimer and ouabain were obtained from Sigma Chemical Co. (St. Louis, MO). PC-1 (serum-free defined culture medium) was obtained from Hycor Biomedical (Irvine, CA). 2,4-dinitrophenylhydrazine (DNP), acetaldehyde, and *trans*-2-nonenal were obtained from Aldrich Chemical Co. (Milwaukee, WI). NO_2 in air at levels of 1 or 5 ppm and compressed air (NO_x ≤0.1 ppm) were obtained from Airgas (Los Angeles, CA). NO_2 levels in air were certified to be of ±5% analytical accuracy for a period of six months from the date of production. For some experiments, NO_2 levels in air were verified with an NO_x analyzer, Model 1150SP (Interscan Corp. Chatsworth, CA). Other reagents were obtained from standard commercial sources.

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Preparation of Tracheobronchial Epithelial Cell Monolayers Cultured in an Air Interface

Male Hartley guinea pigs [specific pathogen-free; Crl:(HA)BR], weighing 250–300 g, were obtained from Charles River (Wilmington, MA). Animals were euthanized with sodium pentobarbital (300 mg/kg) given intraperitoneally. Tracheobronchial epithelial cells were isolated following established procedures (*16–20*). Isolated airway epithelial cells were resuspended in PC-1 medium (*16–20*), which was supplemented with 2 mM L-glutamine, 100 U penicillin G/ml, 100 µg streptomycin/ml, 0.25 µg amphotericin B/ml, and 50 µg gentamicin/ml. GPTE cells were plated at a density of 1.0 x $10^6/cm^2$ in 6.5- or 12-mm collagen-treated Transwells (Costar, Cambridge, MA) (*16–19*). Cells were allowed to adhere to the collagen-treated membrane substratum for 24 hr (*16–19*). On day 1 (24 hr), basolateral and apical media were removed. To grow cells in an air interface, 0.4 or 0.9 ml PC-1 medium was subsequently added only to the basolateral side of the cell monolayer for 6.5- or 12-mm Transwells, respectively (*16–19*). Everyday thereafter, fluid on the basolateral surface was replenished with PC-1 culture medium while the apical surface was essentially left nominally fluid free (*16–19*).

Exposure to Nitrogen Dioxide

Monolayers with similar spontaneous potential difference (SPD) and transepithelial resistance (R_{TE}) values, as measured with the use of a Millicell ERS (Millipore, Bedford, MA), were paired into air or NO_2 exposure groups prior to actual exposure (*18*). Confluent GPTE monolayers in 12-mm Transwells were rinsed twice with 2 ml (0.5 ml added to the apical side and 1.5 ml to the basolateral side) of Krebs Ringers phosphate-buffered HEPES solution (KRPH) composed of 125 mM NaCl, 5 mM KCl, 1.0 mM $MgSO_4$, 8.5 mM Na_2HPO_4, 1.4 mM NaH_2PO_4 , 5 mM glucose, 1.3 mM $CaCl_2$, and 10 mM HEPES, pH 7.4, at 37°C (*18,19*). Following the two rinses, KRPH (0.9 ml) was added to the basolateral side of the monolayer, while the apical side was left fluid free to facilitate contact with air or NO_2. Monolayers in 6.5-mm Transwells were rinsed twice with 1.25 ml (0.25 ml added to the apical side and 1.0 ml to the basolateral side) of KRPH and then 0.4 ml was added to the basolateral side alone. Monolayers were allowed to equilibrate in KRPH for 60 min at 37°C (*18,19*). GPTE monolayers were then exposed to NO_2 (1 or 5 ppm) in air or air alone using previously described procedures (*18,19*). Nominal NO_2 levels rather than actual levels have been provided; exposure time was 1 hr.

Identification and Measurement of DNP-carbonyl Compounds

Basolateral fluid or apical rinse fluid containing products liberated from exposed GPTE monolayers were derivatized with DNP using procedures previously described by Eserbauer et al. (*21*) and Poli et al. (*22*), as modified by Robison et al. (*23*). DNP-carbonyl compounds were separated into classes by thin layer chromatography, and individual aldehydes were quantitated through HPLC separation in conjunction with a Perkin Elmer LC 235 UV diode array detector (Perkin Elmer, Norwalk, CT). The HPLC eluate was monitored at 235 and 365 nm. Known amounts of 2-decadienal dinitrophenylhydrazone were added as an internal standard for quantitation of DNP-derivatized carbonyl compounds. The concentrations of DNP derivatives were estimated by UV analysis at 365 nm using $\varepsilon = 2.8 \times 10^4\ M^{-1}cm^{-1}$ at 365 nm (*21*). The concentration of the Di-DNP (Di-dinitrophenylhydrazine) derivative of glycolaldehyde was determined using $\varepsilon = 4.14 \times 10^4\ M^{-1}cm^{-1}$ at 365 nm (personal communication, M. Thomas).

Effect of Glycolaldehyde on Airway Epithelial Bioelectric Properties

Airway epithelial viability in response to glycolaldehyde treatment was evaluated through measurements of bioelectric properties (short circuit current and R_{TE}). Monolayers were mounted in Ussing chambers (Precision Instrument Design, Tahoe City, CA) and allowed to equilibrate for 15 min in 10 ml of KRPH on both sides (*18*). Glycolaldehyde was added to either the apical or basolateral bathing fluid at concentrations ranging from 0.1 to 20 mM. Short circuit current (SCC) was measured with an automatic voltage/current clamp (DVC-1000; World Precision Instruments, Sarasota, FL) (*18*). The monolayer was continuously short circuited except for a brief period every 30 sec when a constant voltage pulse (dV = 2 mV) was imposed for 3 sec to yield a current response (dI) (*18*). R_{TE} was estimated from the relation of dV/dI and SPD estimated from R_{TE} x SCC (*18*). Bioelectric properties were monitored for 30 min following addition of glycolaldehyde.

Measurement of Airway Epithelial Ouabain-sensitive ^{86}Rb Uptake

To estimate the Na^+,K^+-ATPase activities of glycolaldehyde-treated airway epithelial monolayers, we measured ^{86}Rb (K^+ surrogate) uptake into epithelial cells from the basolateral fluid in the presence and absence of 1 mM ouabain in the basolateral fluid using procedures previously described by Robison and Kim (*19*) and Kim and Suh (*24*). GPTE monolayers, in 6.5-mm Transwells, were treated basolaterally with glycolaldehyde (1 to 20 mM) or the vehicle control (water) for 15 min. After the 15-min treatment, monolayers were separated into two groups. Ouabain (1 mM) was added to the basolateral fluid of one group of monolayers for a 30-min incubation at 37°C, while the other group of monolayers received an equal volume of vehicle (i.e., 25 µl water) added to the basolateral KRPH (400 µl) (*19*). Following ouabain or vehicle treatment, $^{86}RbCl$ was added to the basolateral fluid (final concentration 10 µCi/ml) and incubated for 5, 15, or 30 min (*19*). Glycolaldehyde was present in the basolateral fluid throughout the ouabain treatment and ^{86}Rb uptake periods. Monolayers were washed, solubilized, and counted for ^{86}Rb activity as previously described (*19*). Apparent Na^+,K^+-ATPase activity was expressed as ouabain-sensitive ^{86}Rb uptake (nanomoles per mg protein per 5, 15, or 30 min). Cell protein was measured using the BCA protein assay (Pierce, Rockford, IL).

Measurement of Specific ^3H-Ouabain Binding

With the use of previously described procedures by Robison and Kim (*19*), specific ^3H-ouabain binding was used to measure alterations of density and affinity of Na^+,K^+-ATPase on the airway epithelial basolateral cell surface in response to glycolaldehyde treatment. Monolayers were treated with glycolaldehyde (0 or 20 mM) for 15 min; basolateral fluid was removed and replaced with ^3H-ouabain binding assay buffer which was composed of 250 mM sucrose, 3 mM $MgSO_4$, 3 mM Na_2HPO_4, 1 mM Na_3VO_4, 10 mM Tris-HCl, and 0.5% BSA at pH 7.2 and containing ^3H-ouabain at concentrations ranging from 0.125 to 2 µM (*19,25–27*). Monolayers, in 6.5-mm Transwells, were allowed to bind ^3H-ouabain for 2 hr at 37°C. Nonspecific binding was determined in the presence of excess (1,000 ×) unlabeled ouabain at each radiolabeled ouabain concentration (*19*). Monolayers were washed, solubilized, and counted as previously described, and alterations of ouabain binding were determined by Scatchard analysis (*19*).

Statistical Analysis

Data are expressed as the mean ± standard error of the mean (SEM). NO_2 and air or glycolaldehyde and control treatment groups were compared using analyses of variance

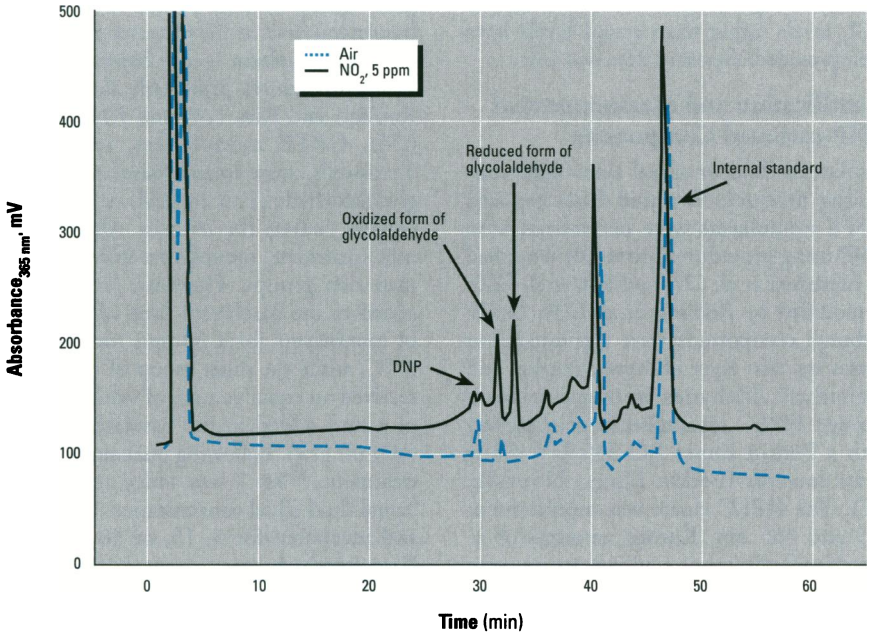

Figure 1. HPLC separation of dinitrophenylhydrazine (DNP) derivatives of carbonyl compounds released into the basolateral fluid from guinea pig tracheobronchial epithelial (GPTE) monolayers exposed for 1 hr to air or 5 ppm NO_2. The DiDNP (Di-dinitrophenylhydrazine) derivatives of glycolaldehyde are present as two peaks: 31.8 min, the oxidized form, which has lost two hydrogens, and 33.3 min, the reduced form. Release of glycolaldehyde toward the apical compartment was minimal in comparison with that toward the basolateral fluid. The DNP derivative of decadienal, 47.2 min, has been added as an internal standard for quantitation.

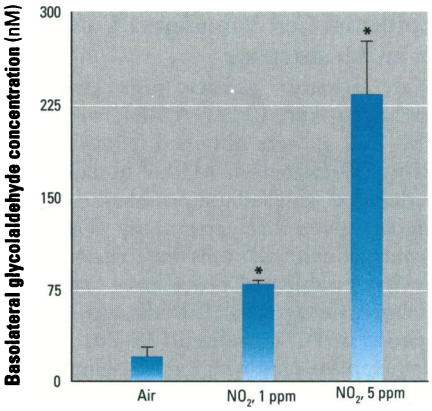

Figure 2. Glycolaldehyde concentrations (nM) in the basolateral fluid following exposure of confluent GPTE monolayers for 1 hr to air or NO_2 (1 or 5 ppm). The oxidized and reduced forms of glycolaldehyde have been summed together to yield a total. Each point represents the mean SEM from three to four monolayers. Asterisks denote a statistical significance of $p \leq 0.05$, compared to the corresponding air controls.

with the Crunch Interactive Statistical Package (Crunch, San Francisco, CA). A comparison of factor means determined to be statistically different was compared with the post hoc Tukey A test. A p value ≤0.05 was considered significant.

Results

Generation of Carbonyl Compounds in Response to NO_2 Exposure

HPLC separation of zone 2 carbonyl compounds indicated that a major aldehydic compound released into the basolateral fluid from NO_2-exposed GPTE monolayers was the Di-DNP derivative of glycolaldehyde (Fig. 1). Derivatization with DNP, TLC separation, and HPLC retention time of this compound generated from GPTE monolayers are consistent with previous identification of glycolaldhyde in this laboratory using identical techniques and confirmation of structure with the use of gas chromatography/mass spectrometry (*23,28*). The Di-DNP derivative of glycolaldehyde was present as two peaks, the first with a retention time of 31.8 min, which was the oxidized form that has lost two hydrogens, and the second at 33.3 min, which was the reduced form (*23*). A DNP derivative of authentic glycolaldehyde was found to co-elute with these two peaks obtained from an extract of the basolateral fluid treated with DNP. The oxidized form of glycolaldehyde was observed in air controls; however, the reduced form was not present. The DNP derivative of decadienal, with a retention time at 47.2 min, was added as an internal standard for quantitation. Glycolaldehyde levels were significantly increased in response to a 1-hr exposure to 1 or 5 ppm NO_2 as compared with air controls (Fig. 2). Release of glycolaldehyde toward the apical side was below the detection limit.

Effect of Glycolaldehyde on Bioelectric Properties

The effects of exogenous glycolaldehyde on GPTE bioelectric properties (e.g., SCC and R_{TE}) were evaluated (Table 1). GPTE monolayers mounted in Ussing chambers were bathed with KRPH on both sides, and glycolaldehyde (2 to 20 mM) was added to the basolateral fluid. Bolus addition of glycolaldehyde at concentrations <1 mM had minimal effects on the SCC or R_{TE}.

Effect of Glycolaldehyde on Ouabain-sensitive ^{86}Rb Uptake

Treatment of the basolateral aspect of GPTE monolayers with 20 mM glycolaldehyde transiently increased ouabain-sensitive ^{86}Rb uptake at 5 min; however, at 15 and 30 min, uptake was significantly decreased below control levels (Fig. 3). Concentrations of 5 or 10 mM did not alter ^{86}Rb uptake at 5 min in comparison with control; however, the uptake at 30 min was significantly decreased.

Table 1. Effect of bolus addition of glycolaldehyde on the bioelectric properties (SCC and R_{TE}) of confluent GPTE monolayers

Treatment	SCC	R_{TE}
Control	100 ± 0	100 ± 0
Glycolaldehyde 2 mM	101.5 ± 3.2	107.3 ± 0.2*
Glycolaldehyde 10 mM	103.8 ± 3.5	105.8 ± 11.1
Glycolaldehyde 20 mM	93.6 ± 3.1	90.5 ± 5.1

Each point represents the mean SEM from three to four monolayers. Values are expressed as a percentage of the control, which was set to 100%. Asterisks denote a statistical significance of $p \leq 0.05$, compared to the corresponding controls. Bioelectric properties at time 0 for 35 monolayers used in this study were as follows: SCC, 8.9 ± 0.3 μA/cm^2; and R_{TE}, 1.64 ± 0.05 kOhm-cm^2.

Effect of Glycolaldehyde on Na^+,K^+-ATPase Density and Affinity

The specific ^3H-ouabain binding capacity (B_{max}) and binding constant (K_d) for basolateral Na^+,K^+-ATPase were measured in response to basolateral glycolaldehyde treatment. A representative Scatchard plot for a 15-min treatment of GPTE monolayers with 0 or 20 mM glycolaldehyde is shown in Figure 4. The B_{max} value for monolayers treated with 20 mM glycolaldehyde at 15.4 ± 1.6 pmol/mg protein was unchanged from the control at 16.4 ± 0.5. The K_d value of 0.78 ± 0.15 μM for GPTE monolayers treated with 20 mM glycolaldehyde was unchanged from the control value of 0.85 ± 0.07 μM. Four binding analyses

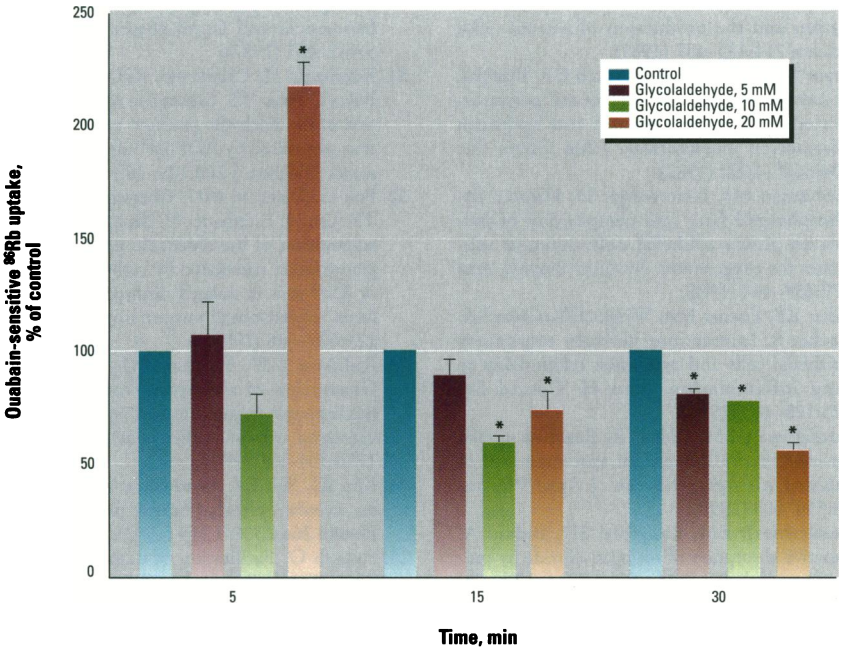

Figure 3. Ouabain-sensitive ^{86}Rb uptake by GPTE monolayers treated for 15 min with glycolaldehyde added to the basolateral fluid at final concentrations of 1, 5, 10, or 20 mM. Each point represents the mean SEM from three monolayers. Data are expressed as nmoles ^{86}Rb/mg protein per 5, 15, or 30 min, respectively. Asterisks denote a statistical significance of $p \leq 0.05$, compared to the corresponding controls. Ouabain-sensitive ^{86}Rb uptake values for control monolayers were as follows: 5 min, 49.2 ± 7.0 nmol ^{86}Rb/mg protein/5min; 15 min, 205.6 ± 15.5 nmol ^{86}Rb/mg protein/15min; and 30 min, 393.3 ± 20.5 nmol ^{86}Rb/mg protein/30min.

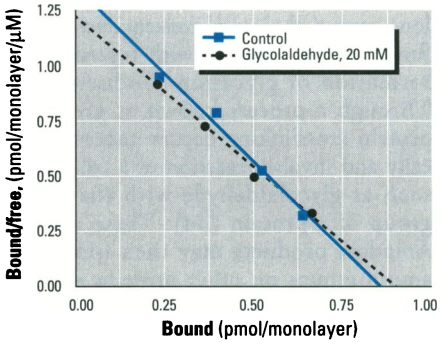

Figure 4. The specific ^3H-ouabain binding capacity (B_{max}) and binding constant (K_d) for basolateral Na^+,K^+-ATPase were measured following treatment with 0 or 20 mM glycolaldehyde added to the basolateral fluid. Representative Scatchard plots are shown. Plots were constructed to determine the number of Na^+,K^+-ATPase/monolayer (abscissa intercept) and the binding constant (negative reciprocal of the slope) determined by linear regression analysis. For the control, K_d = 0.62 µM and B_{max} = 0.85 pmol/monolayer or 14.2 pmol/mg protein. For 20 mM glycolaldehyde, K_d = 0.73 µM and B_{max} = 0.89 pmol/monolayer or 14.9 pmol/mg protein. There were no significant changes in K_d or B_{max} following glycolaldehyde treatment.

were performed for the control and four for 20 mM glycolaldehyde.

Discussion

In the present study, a significant generation of aldehydes from airway epithelial monolayers exposed to NO_2 was detected. Glycolaldehyde, an α-hydroxyaldehyde, was a major aldehydic product generated from exposure of GPTE monolayers to NO_2. Glycolaldehyde was also identified as a major aldehydic product released from alveolar macrophages exposed to NO_2 (*23,28*). Preliminary studies using ^3H-arachidonic acid-labeled monolayers suggest that glycolaldehyde may be derived from cellular lipids. A recent report has identified a number of α-hydroxyaldehydes derived from peroxidation of arachidonic acid (*29*). Glycolaldehyde may be formed from PUFA through a similar oxidative mechanism. Another possible route for the formation of glycolaldehyde may be through the decomposition of sugars (*5,30,31*); however, the reactivity of NO_2 with sugars is known to be much slower than that found with PUFA. Glycolaldehyde is reactive with proteins, rapidly forming Schiff base adducts with amino groups (*14,15*). These Schiff base adducts can undergo Amadori rearrangement to yield a new aldehyde moiety that can form a Schiff base adduct with another amino group (*14*). This process can modify the physical properties of proteins as well as generate intra- and intermolecular crosslinking in or between proteins, respectively (*14*).

Basolateral glycolaldehyde concentrations estimated in the present studies may be lower than cellular concentrations due to diffusion of this compound into the larger volume of the extracellular medium, as well as losses due to binding with cellular components, metabolism, and volatility (*23*). In our preliminary studies, treatment of GPTE monolayers with exogenous hexanal yielded a recovery of only 17%. Based upon a measure of approximately 5×10^5 cells per confluent monolayer, the total cellular volume is estimated to be about 0.5 µl. Thus, the cellular glycolaldehyde concentration for a 1-hr exposure to 5 ppm NO_2 was calculated to reach a maximum of approximately 3 mM. Assuming continuous generation of glycolaldehyde during the 60-min exposure, the production rate could have been as much as 0.05 mM/min.

The effect of exogenous glycolaldehyde on GPTE ouabain-sensitive basolateral Na^+,K^+-ATPase was examined using approximated cellular concentrations of this compound. Treatment with 20 mM glycolaldehyde, transiently stimulated ouabain-sensitive ^{86}Rb uptake at 5 min; however, uptake was inhibited at 30 min with concentrations ≥5 mM. Changes of airway epithelial bioelectric properties (R_{TE} and SCC) in response to glycolaldehyde treatment were small and do not suggest a major loss of cell viability. The inhibition of ouabain-sensitive ^{86}Rb uptake in response to glycolaldehyde treatment was not associated with any alterations of ouabain binding characteristics, suggesting that aldehyde binding directly to Na^+,K^+-ATPase was either not involved in the observed changes of activity or occurs at sites distant from ouabain binding. These data indicate that the effects of exogenous glycolaldehyde differ from that observed with a 1-hr exposure to 1 or 5 ppm NO_2 where a twofold increase of ouabain-sensitive ^{86}Rb uptake was observed for 2 hr postexposure (*19*) and glycolaldehyde was presumably generated at a continuous low level. The dynamics associated with bolus addition of glycolaldehyde appear to be quite different from those of cellular glycolaldehyde generation in response to NO_2 exposure. These differences may include alterations in access to essential cellular targets, binding to nonessential targets such as the collagen matrix, and metabolism by various cellular enzymes such as aldehyde dehydrogenase (*32,33*). These results with exogenous glycolaldehyde may suggest that other lipid peroxidation products are probably mediating the actions of this reactive gas at levels associated with elevated indoor exposure.

Glycolaldehyde may have significant long-term pathological consequences to cell function and viability with regard to the formation of glycation products (*34*). Through a process known as glycation, protein crosslinking occurs nonenzymatically and involves reaction of a compound such as glycolaldehyde with the amino group of a protein (*34*). These reactive Amadori products may then bind with amino groups on other proteins to form advanced glycation end products (AGEs) through intermolecular crosslinks (*34*). This process may interfere with cell-to-cell attachment as well as cell adhesion to the basement membrane, potentially resulting in significant disruption of the epithelial barrier and development of increased airway reactivity.

In summary, we have found that glycolaldehyde, an aldehydic compound, is generated in elevated levels from airway epithelial monolayers acutely exposed to NO_2, in the range associated with elevated indoor levels. Exogenous glycolaldehyde does not appear to play a significant role in the previously observed NO_2-induced enhancement of airway epithelial Na^+,K^+-ATPase activity, suggesting that the alteration of pump activity is most likely mediated by other lipid peroxidation products yet to be identified. Glycolaldehyde may have significant long-term pathological consequences with regard to disruption of the epithelial barrier through its known reactivity with cellular proteins.

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